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REVIEW

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Unlocking the diagnostic potential of extracellular vesicles in cancer

Milica Popović
University of Belgrade-Faculty of Chemistry
la_bioquimica@chem.bg.ac.rs

Abstract
Extracellular vesicles (EVs) are nanoscale lipid bilayer particles that are secreted by virtually all cells into biofluids; their cargo of nucleic acids, proteins, lipids, and glycans is tumor type and status informative and can be measured via minimally invasive liquid biopsy. Herein, we evaluate critically the EV-based assay diagnostic accuracy in both solid and hematologic malignancies, particularly with regard to exemplar biomarker panels and single-part analytics for pancreatic, prostate, breast, lung, and colorectal cancers as well as hematologic disorders. We emphasize newer immune- and affinity-directed isolation/phenotyping technologies that are more selective and scalable than conventional ultracentrifugation—e.g., microarray capture (EV Array), EV enrichment from cancer cells, magnetic nanopore capture, and filtration/thermophoretic routes—along with single-EV readouts for increased informational content. We also put EV diagnostics in a complementary role to established liquid biopsy modalities (e.g., ctDNA/cfDNA) to maximize detection and disease monitoring, rather than as alternatives. Finally, we outline theranostic applications, including engineered EVs as delivery vehicles and stromal EVs as therapy resistance biomarkers and mediators, to bridge diagnostics with interventional strategies. This roadmap centers clinically meaningful use cases and methodological stringency to drive translation of EV assays into oncology practice
Keywords: extracellular vesicles, cancer, biomarkers, liquid biopsy.

Introduction

Extracellular vesicles (EVs) are a diverse group of membranous nano-sized structures secreted by cells into the extracellular space. According to the manner of biogenesis EVs can be classified into exosomes, microvesicles and apoptotic bodies. (1)Exosomes typically range in size from 30 to 150 nm. They are derived from the endosomal pathway more specifically by inward budding of the membrane of the early endosomeand formation of intraluminal vesicles (ILV). The content of ILVs is carefully sorted and packed into multivesicular bodies (MVB)(2). MVB fuse with the plasma membrane to release exosomes into the extracellular space. Microvesicles are formed by direct outward budding of plasma membrane. Their typical size ranges from 100 nm to 1 µM. The biogenesis mechanism involves trafficking of molecular cargo to the plasma membrane, a redistribution of membrane lipids, and the use of contractile machinery at the surface to allow for vesicle pinching.(3) Apoptotic bodies are released by dying cells in the final stage of apoptosis. Compared to the other extracellular vesicles, the diameter of apoptotic bodies is the largest from 50nm to 5µm. (4). EVs have been identified as important signaling mediators in a myriad of physiological and pathological processes. EVs carry biomolecules from one cell to another, exposing the molecular state of the cell of origin. EVs are emerging as powerful tools for theranostic purposes as their sheer presence in biological fluids designates them as novel and easily accessible sources for the diagnosis of diseases, prognosis and treatment monitoring. (5)

This report prioritizes EV-based diagnostics with translational significance to across salient tumor types (pancreatic, prostate, breast, lung, colorectal) and hematologic settings, and across biofluids including plasma/serum and urine, where biomarker validity, analytical performance, and clinical pragmatism are the priority (6, 7). EV biology and interactions with the tumor microenvironment are considered only in contexts directly relevant to diagnostic interpretation and clinical application—for example, stromal EV–mediated resistance mechanisms that overlap with monitoring use cases.(8, 9)

EV assays are compared and contrasted with circulating tumor DNA/cell-free DNA approaches to establish complementarity in detection and disease dynamics, particularly for risk stratification and longitudinal monitoring(10, 11). The manuscriptstresses scalable, selective isolation and single-particle analytics suitable for clinical pipelines, and we underscore open methodological reporting as a doorway to reproducibility and meta-analysis across laboratories (12).

Role of EVs in cancer

EVs play a significant role in cancer development, progression, and metastasis by facilitating cross-communication between tumor cells and their environment. EVs carry diverse cargo such as proteins, nucleic acids, lipids, and metabolites acting like messengers thereby enabling tumorigenesis, tumor growth, metastasis and drug resistance.

Cancer cell-derived EVs harbor oncogenic molecules like proteins, DNA fragments, and most forms of non-coding RNA that influence the initial hallmarks of cancer such as proliferation, invasion, immune evasion, and drug resistance. Modulation of the tumor microenvironment toward malignancy is one of the key functions of EVs. They initiate angiogenesis by transporting pro-angiogenic factors like VEGF and IL-8 to endothelial cells, promoting vascular remodeling and delivery of nutrients to tumors(13).

In metastasis, EVs are key for tumor-organotropism as they prepare far-away tissues for the colonization of tumor cells. They reprogram immune and stromal cells to create a permissive pre-metastatic niche, typically by delivering integrins, metalloproteinases, and miRNAs that re-organize the extracellular matrix and suppress local immune surveillance (14). Additionally, EVs are the main immune escape mediators; e.g., cancer-derived EVs can carry PD-L1 or Fas ligand to inhibit T-cell activation and induce apoptosis in cytotoxic lymphocytes, creating an immunosuppressive tumor microenvironment (15).

One of the crucial and better-documented roles of EVs in cancer is related to therapeutic resistance. Drug-resistant cancer cells secrete EVs that contain multidrug resistance proteins (e.g., P-glycoprotein), anti-apoptotic proteins, and resistance-predictive microRNAs (e.g., miR-21, miR-222). These vesicles can be internalized by drug-sensitive neighboring cells, spreading resistance features to the tumor cell population. Moreover, EVs can modulate drug metabolizing pathways, efflux pump expression, and DNA repair mechanisms of target cells, rendering the drugs less effective. Some EVs also act as decoys since they bind and sequester therapeutic antibodies or chemotherapeutic drugs, hence diminishing their availability at the tumor site. This multiparameter ability of EVs in resistance not only boosts tumor survival under therapeutic stress but also complicates clinical cancer treatment (16). Thus, EVs are not only products of cancer cells, but also active components involved in mediating tumor progression, metastasis, and drug resistance.

EVs as cancer biomarkers

EVs are promising candidates for cancer diagnosis as a rich source of biomarkers due to their high abundance, stability, and ability to transport dense cargo of tumor-associated molecules. In nearly all bodily fluids, EVs encapsulate proteins, lipids, DNA, and various types of RNAs that are reflective of the molecular profile of their cell of origin, e.g., cancer cells (13), making them a studied source of biomarkers for non-invasive liquid biopsies. EVs are released early during tumorigenesis, often before lesions or metastasis become apparent, which makes it possible to detect them in the early stages (17). It has been demonstrated that specific EV cargo—specifically, miRNAs (e.g., miR-21, miR-10b), long non-coding RNA, and glypican-1 (GPC1) proteins—can distinguish between cancer patients and healthy individuals with excellent sensitivity and specificity. In pancreatic cancer, EVs that are positive for GPC1 and EV-associated miR-21 and miR-1246 have sensitivity of up to 84% and specificity of up to 89% (18, 19, 20). EVs level of certain micro RNAs can offer a more a sensitive tool for stratification of and screening of cancer patients (PCa). miR-21 has been associated with prostate cancer, however its level in EVs may serve as a more accurate noninvasive prognostic biomarker compared with the whole plasma miR-21 for active monitoring of PCa patients (21).

EV-based biomarkers have been evaluated across several cancer types and biofluids, with assays ranging from RNA and lipid profiling to single-particle phenotyping. These studies demonstrate diagnostic accuracy, stratification potential, and monitoring capability in both preclinical and clinical settings (6, 7, 12, 22, 23, 24, 25, 26, 27, 28, 29, 30).

Representative examples are summarized in Table 1, organized by cancer type, EV source, analyte, methodology, and reported performance.

Isolation and Reporting for Clinical Translation

Despite significant progress in the field, major challenges including the standardization of EV isolation, cargo heterogeneity, and pre-analytical variability still need to be addressed before routine clinical implementation can be achieved (32). Present EVs isolation approaches are very inefficient, time-consuming, and expensive. They are generally based on ultracentrifugation, ultrafiltration, precipitation, or immunoaffinity-based exosome isolation. The gold standard in EVs purification is ultracentrifugation, especially with density gradient. However, it is very difficult to standardize all of the ultracentrifugation parameters that can affect the quality of isolated material and in this sense, it is nearly impossible to set up a universally standardized protocol. Furthermore, the variability among sample populations from the same cells and especially different sources renders this standardization even more difficult. Ultracentrifugation is a low-throughput method and often samples, especially from plasma are contaminated with lipoproteins, protein aggregates and other particles of similar size or density making them incompatible with clinical

Table 1. Examples of EV-derived biomarkers across cancer types and their diagnostic performance.

Cancer	EV Source	Analyte(s)	Method	Performance	Cohort/ Model
Pancreatic	Plasma EVs	11 miRNAs	RNA-seq + ML using magnetic nanopore-isolated EVs	88% accuracy in three-way classification (healthy vs PanIN vs PDAC)	PDAC GEMM, train n=27; blinded validation n=57 (31)
Pancreatic	Plasma EVs	EGFR/ marker-defined EV subset	qSMLM single-particle characterization	Identified a PDAC-enriched EV population in patient plasma	Patient samples + cell line benchmarking (25)
Breast (TNBC)	Plasma EVs	Glycan signature	EVLET (lectin + thermophoresis)	91% diagnostic accuracy; 96% longitudinal therapy monitoring	Pilot TNBC cohort (26)
Breast (all subtypes)	Plasma EVs	Lipidome (sphingo-/glycerophospho lipids)	Untargeted lipidomics	Overall 93.1% acc.; 95% primary vs healthy; 89.5% primary vs metastatic	Patients (primary and metastatic) (28)
Breast (preclinical)	Cell/Plasma EVs	Label-free spectral fingerprints	SPARTA Raman (single-particle)	>95% sensitivity/specificity; subtype discrimination	Preclinical/EV isolates(29)
Prostate	Plasma EVs	EV-GDF15 (proteome)	TiO2chemical affinity + MS	AUC 0.908 PCa vs BPH; enhances PSA performance	Multicenter; discovery + independent validation n=457 (22)
Prostate	Urine EVs	EV miRNAs (panel feasibility)	LF-bis-MPA magnetic nanocomposites	Significant EV-miRNA dysregulation in PCa vs controls; rapid, high yield isolation	Urine from PCa (n=20), BPH (n=10), healthy (n=10) (23)
Prostate (bone mets)	Plasma EVs	EV-CDCP1 (surface protein)	Functional + clinical association	Elevated in bone metastatic PCa; facilitator of osteoclastogenesis	Patient plasma + mechanistic assays (30)
Colorectal	Serum/Plasma sEVs	Multi-omic cargo overview	Synthesis of recent CRC sEV studies	Promising biomarker space; isolation/reporting challenges	State-of-the-art review (7)
Hematologic (AML)	MSC-EVs	Functional modulation of AML	In vitro functional assays	Dose-dependent anti-proliferative/apoptotic effects; supports therapeutic/biomarker potential	AML cells + ML classifier on EV data (27)

utilization. There is emerging experimental evidence that substantial amounts of EVs RNA and proteins are lost during ultracentrifugation. Additionally, using ultracentrifugation as a method of choice makes scaling-up extremely difficult if not completely unobtainable. Immuno-affinity-based techniques offer an advantage of selecting pathology-specific EVs and preserving them intact. Unfortunately, elevated costs and time constraints related to production of monoclonal antibodies, as well as their large dimensions render them inappropriate for scalable and high-throughput EV isolation. Recent scientific endeavors are aimed at developing versatile, cost-effective methodologies for scalable isolation (µL to mL; mL to >L) of high-purity EVs from bio-samples, which are urgently needed to open new perspectives in EVs-based theranostics. Novel methods for EV isolation based on single-domain antibodies as selective agents have been proven effective for various biological sources such as plasma and urine (33, 34, 35).

Table 2. Comparison of EV isolation and capture platforms relevant for clinical translation.

Platform	Capture principle	Time	Throughput/ Scalability	Specificity	Downstream compatibility	Clinical readiness notes
TiO2chemical affinity	Phosphate/ acidic lipid affinity for broad EV capture	~1–2 h	High; plates/batches; robust	Broad EVs from plasma	Deep proteomics/ MS	Cohort-scale reproducibility; enabled EV-GDF15 discovery/validation (22)
EV Array (microarray)	Multiplex antibody capture (CD9/63/81 + targets)	3–4 h	Slides/96-well; multiplex	Tetraspanin+ EV phenotyping	Relative quantitation; small volumes	Detects from unpurified plasma; high sensitivity (38)
Magnetic nanopore	Nanopore + magnetic selection	<1 h	Arrayable	Subpopulation enrichment	RNA-seq + ML	Identified miRNA panel discriminating PDAC states (12)
EV-FISHER (MOF + cleavable lipid probe)	Cholesterol probe capture with enzymatic release	~40 min	Bench-scale	Broad EV capture; GPC-1 profiling	Flow cytometry/ single EV	Demonstrated improved yield over UC; representative of MOF workflows (12)
CD147 immunocapture	Antibody capture of CD147+ EVs	1–2 h	Beads/flow platforms	Enriches cancer cell-derived, miRNA-rich EVs	miRNA assays; sequencing	Boosts detection of tumor miRNAs vs conventional methods (24)
LF-bis-MPA-MNPs	Lactoferrin-decorated magnetic NPs	~1 h	Magnetic; scalable	Electrostatic + biorecognition	RT-qPCR miRNAs	Rapid urinary EV enrichment; point-of-care promise (23)
IBMsv MOF-bubble + SERS	Buoyancy MOF corona + multiplex SERS nanotags	~2 min	Portable; high throughput	Multiplex surface markers	Single-EV phenotype	>95% accuracy across five cancers; response monitoring

To enable translational use of EVs as biomarkers, multiple platforms have been developed for their isolation and capture. These methods vary in throughput, specificity, and downstream compatibility, and are at different levels of clinical readiness (36, 37). A comparative overview of key platforms is provided in **Table 2**, highlighting their capture principles, analytical potential, and performance notes derived from recent clinical and preclinical studies (12, 22, 23, 31, 38, 39).

The isolation of extracellular vesicles is a juncture point in any diagnostic pathway because the chosen approach determines recovery, purity, scalability, and ultimately the credibility of any subsequent biomarker claim (36, 37). Conventional techniques such as differential ultracentrifugation remain prevalent; however, they lead to heterogenous particle populations and co-purification of plentiful lipoproteins and protein aggregates that can mask lower-abundance, disease-related cargo, and their inconsistent performance within laboratories—variability in g-forces, rotor k-factors, and washing cycles—avoids comparability and reproducibility among studies (36, 40). These problems are matrix-related in urine, where uromodulin/Tamm–Horsfall protein polymers can entrap vesicles, lower yield, and make proteomic readouts difficult unless actively broken and removed during processing (41). With growing recognition that method rigor reporting is as important as the biomarker signals themselves, community resources such as EV-TRACK and its EV-METRIC have accentuated the frequency of suboptimal reporting and provided concrete checklists for isolation parameters, particle characterization, and quality controls to enable site validation and meta-analysis (36), as part of the broader tendency towards structured framework reporting in biomedicine to make transparency and peer review more efficient (42).

To counter the disadvantage of bulk physical separation, many strategies of affinity-guided capture have been created that facilitate direct improvements in selectivity, velocity, and scalability for clinical application (Table 2). Chemical affinity capture in plate formats leverages EV surface chemistry to standardize binding and elution across large plasma cohorts, thereby allowing for reproducible deep proteomics and discovery-to-validation pipelines for protein biomarkers with diagnostic potential while allowing direct integration with orthogonal liquid biopsy readouts such as circulating tumor DNA in multivariable models (37). Immunocapture takes this specificity even further via enrichment of diagnostically significant subpopulations: CD147 recognizes an EV class that is biogenetically distinct from classic tetraspanin-positive EVs and is selectively loaded with miRNA cargo by hnRNPA2/B1, with signal derived predominantly from cancer cells in xenograft models; separation of circulating miRNAs by CD147 immunocapture increases detection sensitivity for tumor-specific miRNAs and better indicates tumor miRNA signatures than conventional bulk separation(24). Simultaneously, microarray antibodies such as the EV Array and miniaturized platforms such as ExoChip demonstrate that capture and readout can be performed directly from unprocessed small volumes of serum or plasma with high analytical sensitivity, thereby reducing pre-analytical handling and stabilizing turn-around time in discovery and triage settings (37, 43).

Physical and nanomaterial-facilitated selection platforms add complementary capability by combining enrichment with functionally relevant downstream analytics (Table 2). Magnetic nanopore capture was utilized to isolate diagnostically informative EV subsets for small-RNA sequencing and machine-learning classification; in a genetically engineered mouse model of pancreatic ductal adenocarcinoma, this approach yielded an eleven-miRNA EV panel that classified healthy, PanIN, and PDAC states with 88% accuracy in blinded validation, offering an early detection proof-of-concept based on subpopulation enrichment (31). Thermophoretic assays combined with optimized filtration also readily translate to clinical environments: an EVLET, lectin-guided thermophoretic protocol enabled fast glycan analysis of plasma EVs and achieved 91% for the detection of triple-negative breast cancer and 96% for longitudinal therapy monitoring in a pilot cohort, underlining the clinical value of EV surface glycomics when purification is co-designed with the resulting readout (26).

Because the composition of biofluids has a direct impact on recovery of EVs and specificity of assays, matrix-corrected optimization is essential for clinical-grade analysis. Disruption of networks of uromodulin polymers in expressed prostatic secretions of urine followed by washing with alkaline condition releases entrapped vesicles and removes co-isolated contaminants and, therefore, permits detailed proteomics analysis of EVs from prostate origin that would otherwise be masked by matrix effects (41). Direct microarray or microfluidic immunocapture from unclarified plasma and serum samples can minimize fractionation-induced variability as long as non-EV protein carryover and lipoprotein contamination are evaluated with suitable negative markers and orthogonal sizing or imaging controls to verify particle identity (36, 40). Beneath such matrix-aware strategies is the recognition that there is no single, universal isolation strategy; rather, method choice must be explicitly matched to biofluid, analyte type, and target clinical application, whether early diagnosis, triage, risk stratification, or monitoring (37).

New capabilities in single-particle analytics now step in as essential complements to isolation through confirmation of enrichment, counting of heterogeneity, and enhancement of informational return per vesicle. Quantitative single-molecule localization microscopy can measure size and biomarker density on individual particles and has demonstrated

that a pancreatic cancer–enriched EV population is present directly in patient plasma, confirming the diagnostically exploitable potential of nanoscale phenotypic heterogeneity when correlated with appropriate capture strategies (25). Label-free single-particle Raman spectroscopy has also shown more than 95% sensitivity and specificity in distinguishing cancer vs. non-cancer EVs and can resolve closely related subtypes of breast cancers, allowing high-granularity phenotyping in label-free formats that are amenable to longitudinal monitoring (29). Thermophoretic glycan profiling provides an orthogonal surface readout that is useful both for response measurement and detection in triple-negative breast cancer, highlighting the value of integrating surface, proteomic, and RNA cargo signals at the single-particle or subpopulation level within a single diagnostic pathway (26).

As part of the larger liquid biopsy framework, EV assays need to be framed as complementary to circulating tumor DNA and cell-free DNA, rather than competitive technologies, because they bear cell-of-origin information in proteins, lipids, glycans, and small RNAs not accessible from ctDNA, and ctDNA is optimized for detecting tumor fraction and tracking genomic evolution over time (10, 11). In metastatic prostate cancer, cfDNA longitudinal tumor fraction is correlated with metastatic burden and response to therapy and provides good utility in disease dynamics that can be augmented by EV-based phenotyping for functional interpretation as well as increased sensitivity in multimodal algorithms (8, 11). Contemporary syntheses emphasize that EV-mediated communication in the tumor microenvironment—e.g., stromal determinants of resistance—is mechanistic context to biomarker alterations, especially when EV diagnostics are used to direct adaptive therapy or to predict resistance(6, 8).

Clinical translation also requires transparency and reproducibility in reporting, and these factors are always acknowledged as determinants of scientific transparency in both the clinical literature and in the EV field itself (40, 42). Authors should explain pre-analytics like sample type, collection tubes, handling temperatures and times, and any clarification steps in a clear manner, with matrix-specific mitigations like uromodulin reduction and alkaline washes explained in urine-based protocols to facilitate replication and quality benchmarking (40, 41). Isolation parameters—operating conditions, method class, and capture chemistries—have to be reported in sufficient detail to enable replication between platforms, and particle identity has to be validated by orthogonal sizing as well as by canonical positive and negative markers suitable to the biofluid and method, according to EV-TRACK guidelines for the completeness of reporting (36, 40). Analytical approaches, calibration and normalization, and validation design should be pre-specified and, to the extent possible, blinded and consistent with the targeted clinical use; these elements reflect current reporting templates for diagnostics and biomarker studies and strongly enhance peer review and reuse (40, 42). Finally, as terminology is itself a moving target with subtypes and biogenesis pathways being revised, authors have to report operational definitions and report limitations in nomenclature to ensure interpretability across studies of differing isolation and characterization depth (44, 45).

In short, the field has long since outgrown differential ultracentrifugation and now comfortably resides on immuno-, chemical-, microfluidic-, and nanomaterial-based platforms that increase yield, purity, and tumor specificity at rates compatible with clinical testing, and that can be supplemented with single-particle analytics to release diagnostically enriched subpopulations with actionable resolution (46). Transparent, EV-TRACK-aligned reporting; matrix-tailored, versioned standard operating procedures; and prespecified plans for multimodal integration with ctDNA and clinical variables now represent the defining steps for translating EV assays from discovery to reproducible, clinically relevant diagnostics with the understanding that EVs report on and shape tumor biology, including response and resistance to therapy in the tumor microenvironment (6, 8)

Conclusion

EVs have become indispensable players in cancer biology, acting not only as cellular activity waste but as dynamic players in tumor development, metastasis, immune evasion, and drug resistance. Their ability to transport a high and diverse payload of biomolecules—characteristic of their cells of origin—is particularly valuable for cancer diagnosis. The enrichment of tumor-related proteins, RNAs, and lipids in EVs, as well as secretion within easily accessible body fluids, positions them well to be effective candidates for efficient non-invasive biomarker identification and early cancer detection. Despite having great potential, there are technical hindrances to their clinical use, particularly with respect to standardization and scalability of EV isolation procedures. Current gold-standard techniques such as ultracentrifugation are time-consuming, wasteful, and not favorable for repeated clinical use. While immunoaffinity-based techniques are specific, scalability by expense and complexity is low. New high-throughput, low-cost EV isolation technologies—e.g., utilization of single-domain antibodies—are a key next step towards unlocking the complete diagnostic and therapeutic potential of EVs. With continued research and resolution of those technology hurdles, EVs can revolutionize cancer diagnostics such that the early detection, enhanced patient stratification, and monitoring of treatment response in real time can be feasible in a minimally invasive manner.

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